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(54) Title: *CORYNEBACTERIUM GLUTAMICUM* GENES ENCODING STRESS, RESISTANCE AND TOLERANCE
PROTEINS

(57) Abstract: Isolated nucleic acid molecules, designated SRT nucleic acid molecules, which encode novel SRT proteins from *Corynebacterium glutamicum* are described. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing SRT nucleic acid molecules, and host cells into which the expression vectors have been introduced. The invention still further provides isolated SRT proteins, mutated SRT proteins, fusion proteins, antigenic peptides and methods for the improvement of production of a desired compound from *C. glutamicum* based on genetic engineering of SRT genes in this organism.

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What is claimed:

1. An isolated nucleic acid molecule from *Corynebacterium glutamicum* encoding a stress, resistance, or tolerance gene, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
2. The isolated nucleic acid molecule of claim 1, wherein said stress, resistance, or tolerance gene is selected from the group consisting of nucleic acid molecules involved in a stress response, tolerance, or resistance to temperature stresses, pH stresses, oxygen stresses, osmotic stresses, toxic chemicals, oxygen radicals, antibiotics, or to lincomycin.
3. An isolated *Corynebacterium glutamicum* nucleic acid molecule selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
4. An isolated nucleic acid molecule which encodes a polypeptide sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
5. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide selected from the group of amino acid sequences consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
6. An isolated nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, or

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a portion thereof, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.

- 5 7. An isolated nucleic acid molecule comprising a fragment of at least 15 nucleotides of a nucleic acid comprising a nucleotide sequence selected from the group consisting of those sequences set forth as odd-numbered SEQ ID NOs of the Sequence Listing, provided that the nucleic acid molecule does not consist of any of the F-designated genes set forth in Table 1.
- 10 8. An isolated nucleic acid molecule which hybridizes to the nucleic acid molecule of any one of claims 1-7 under stringent conditions.
- 15 9. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1-8 or a portion thereof and a nucleotide sequence encoding a heterologous polypeptide.
10. A vector comprising the nucleic acid molecule of any one of claims 1-9.
- 20 11. The vector of claim 10, which is an expression vector.
12. A host cell transfected with the expression vector of claim 11.
13. The host cell of claim 12, wherein said cell is a microorganism.
- 25 14. The host cell of claim 13, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.
15. The host cell of claim 12, wherein the expression of said nucleic acid molecule results in the modulation in production of a fine chemical from said cell.
- 30 16. The host cell of claim 15, wherein said fine chemical is selected from the group consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine

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and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides, and enzymes.

- 5 17. A method of producing a polypeptide comprising culturing the host cell of claim 12 in an appropriate culture medium to, thereby, produce the polypeptide.
18. An isolated stress, resistance, or tolerance polypeptide from *Corynebacterium glutamicum*, or a portion thereof.
- 10 19. The protein of claim 18, wherein said stress, resistance, or tolerance polypeptide is selected from the group consisting of proteins involved in a stress response, tolerance, or resistance to temperature stresses, pH stresses, oxygen stresses, osmotic stresses, toxic chemicals, oxygen radicals, antibiotics, or to lincomycin.
- 15 20. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.
- 20 21. An isolated polypeptide comprising a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, or a portion thereof, provided that the amino acid sequence is not encoded
- 25 by any of the F-designated genes set forth in Table 1.
22. The isolated polypeptide of any of claims 18-21, further comprising heterologous amino acid sequences.
- 30 23. An isolated polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 50% homologous to a nucleic acid selected from the group consisting of those sequences set forth as odd-numbered SEQ ID

NOs of the Sequence Listing,, provided that the nucleic acid molecule does not consist of any of the F-designated nucleic acid molecules set forth in Table 1.

24. An isolated polypeptide comprising an amino acid sequence which is at least 50% homologous to an amino acid sequence selected from the group consisting of those sequences set forth as even-numbered SEQ ID NOs of the Sequence Listing, provided that the amino acid sequence is not encoded by any of the F-designated genes set forth in Table 1.

25. A method for producing a fine chemical, comprising culturing a cell containing a vector of claim 12 such that the fine chemical is produced.

26. The method of claim 25, wherein said method further comprises the step of recovering the fine chemical from said culture.

27. The method of claim 25, wherein said method further comprises the step of transfecting said cell with the vector of claim 11 to result in a cell containing said vector.

28. The method of claim 25, wherein said cell belongs to the genus *Corynebacterium* or *Brevibacterium*.

29. The method of claim 25, wherein said cell is selected from the group consisting of: *Corynebacterium glutamicum*, *Corynebacterium herculis*, *Corynebacterium lilium*, *Corynebacterium acetoacidophilum*, *Corynebacterium acetoglutamicum*, *Corynebacterium acetophilum*, *Corynebacterium ammoniagenes*, *Corynebacterium fujiokense*, *Corynebacterium nitrilophilus*, *Brevibacterium ammoniagenes*, *Brevibacterium butanicum*, *Brevibacterium divaricatum*, *Brevibacterium flavum*, *Brevibacterium healii*, *Brevibacterium ketoglutamicum*, *Brevibacterium ketosoreductum*, *Brevibacterium lactofermentum*, *Brevibacterium linens*, *Brevibacterium paraffinolyticum*, and those strains set forth in Table 3.

30. The method of claim 25, wherein expression of the nucleic acid molecule from said vector results in modulation of production of said fine chemical.
31. The method of claim 25, wherein said fine chemical is selected from the group
5 consisting of: organic acids, proteinogenic and nonproteinogenic amino acids, purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated and unsaturated fatty acids, diols, carbohydrates, aromatic compounds, vitamins, cofactors, polyketides and enzymes.
- 10 32. The method of claim 25, wherein said fine chemical is an amino acid.
33. The method of claim 32, wherein said amino acid is drawn from the group consisting of: lysine, glutamate, glutamine, alanine, aspartate, glycine, serine, threonine, methionine, cysteine, valine, leucine, isoleucine, arginine, proline, histidine,
15 tyrosine, phenylalanine, and tryptophan.
34. A method for producing a fine chemical, comprising culturing a cell whose genomic DNA has been altered by the inclusion of a nucleic acid molecule of any one of claims 1-9.
- 20 35. A method for diagnosing the presence or activity of *Corynebacterium diphtheriae* in a subject, comprising detecting the presence of one or more SEQ ID NOs 1 through 304 of the Sequence Listing in the subject, provided that the sequences are not or are not encoded by any of the F-designated sequences set forth in Table 1, thereby
25 diagnosing the presence or activity of *Corynebacterium diphtheriae* in the subject.
36. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the nucleic acid molecule is disrupted.
- 30 37. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set forth as odd-numbered SEQ ID NOs of the

Sequence Listing , wherein the nucleic acid molecule comprises one or more nucleic acid modifications from the sequence set forth as odd-numbered SEQ ID NOs of the Sequence Listing .

- 5 38. A host cell comprising a nucleic acid molecule selected from the group consisting of the nucleic acid molecules set as odd-numbered SEQ ID NOs of the Sequence Listing, wherein the regulatory region of the nucleic acid molecule is modified relative to the wild-type regulatory region of the molecule.

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Gln Asp Asp Asp Asp Phe Ile Gln Pro Gly Ile Leu Tyr Arg Glu Val
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 Pro Leu Phe Gly Ile Ala Asn Gln Leu Leu Ala Ala Ile Ala Leu Ala
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 Leu Val Leu Val Val Val Val Lys Lys Gly Leu Tyr Lys Trp Ala Trp
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 Ser Trp Gln Lys Ile Phe His Ser Asp Pro Ala Ile Gly Tyr Trp Ala
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 Gln Asn Ala Asn Phe Arg Asp Ala Lys Ser Gln Gly Leu Thr Glu Phe
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 Gly Ala Ala Lys Ser Pro Glu Ala Ile Asp Ala Val Ile Arg Asn Thr
 675 680 685
 Met Ile Gln Gly Ile Leu Ser Ile Leu Phe Ala Val Leu Val Leu Val
 690 695 700
 Val Val Gly Ala Ala Ile Ala Val Cys Ile Lys Ser Ile Arg Ala Arg
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 Ala Ala Gly Thr Pro Leu Glu Thr Thr Glu Glu Pro Asp Thr Glu Ser
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 Glu Phe Phe Ala Pro Thr Gly Phe Leu Ala Ser Ser Arg Asp Lys Glu
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